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      Canine mitochondrial uncoupling proteins: structure and mRNA expression of
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      three isoforms in adult beagles.
      Ishioka Katsumi; Kanehira Katsushi; Sasaki Noriyasu; Kitamura Hiroshi;
 ΑU
      Kimura Kazuhiro; Saito Masayuki
      Laboratory of Biochemistry, Department of Biomedical Sciences, Graduate
 CS
      School of Veterinary Medicine, Hokkaido University, 060-0818, Sapporo,
      Japan.
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      Journal code: 9516061. ISSN: 1096-4959.
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     Palmieri L; Pardo B; Lasorsa F M; del Arco A; Kobayashi K; Iijima M;
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     Runswick M J; Walker J E; Saheki T; Satrustegui J; Palmieri F
     Department of Pharmaco-Biology, University of Bari, Via Orabona 4, 70125
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     Bari, Italy.
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     2002110256 MEDLINE
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     21833809 PubMed ID: 11845285
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     Rapid decrease of RNA level of a novel mouse mitochondria solute carrier
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     protein (Mscp) gene at 4-5 weeks of age.
     Li Q Z; Eckenrode S; Ruan Q G; Wang C Y; Shi J D; McIndoe R A; She J X
ΑU
     Department of Pathology, Immunology and Laboratory Medicine, Box 100275,
CS
     Center for Mammalian Genetics, and Diabetes Center of Excellence, College
     of Medicine, University of Florida, Gainesville, Florida 32610, USA.
     1P01AI-42288 (NIAID)
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     UCP5 and therapeutic uses
     Adams, Sean; Pan, James
 IN
     Genentech, Inc., USA
 PA
     PCT Int. Appl., 90 pp.
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     proteins in wheat: wheat UCP genes are not regulated by low temperature
     Murayama, S.; Handa, H.
AU
     Laboratory of Plant Genecology, Hokkaido National Agricultural Experiment
CS
     Station, Sapporo, 062-8555, Japan
     Molecular and General Genetics (2000), 264(1-2), 112-118
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     99299208 PubMed ID: 10369894
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     long-chain fatty acids, encodes a putative homologue of the mammalian
     carnitine/acylcarnitine carrier.
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ΑU
     Departamento de Microbiologia y Parasitologia, Facultad de Farmacia,
CS
     Universidad de Alcala, Ctra. Madrid-Barcelona Km 33, E-28871 Alcala de
     Henares (Madrid), Spain.
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     Assessment of uncoupling activity of the human uncoupling protein 3 short
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     form and three mutants of the uncoupling protein gene using a yeast
     heterologous expression system.
     Hagen T; Zhang C Y; Slieker L J; Chung W K; Leibel R L; Lowell B B
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     Department of Medicine, Beth Israel Deaconess Medical Center and Harvard
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      Medical School, Boston, MA 02215, USA.
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      FEBS LETTERS, (1999 Jul 9) 454 (3) 201-6.
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      99295931 PubMed ID: 10369257
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      The gene mutated in adult-onset type II citrullinaemia encodes a putative
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    Kobayashi K; Sinasac D S; Iijima M; Boright A P; Begum L; Lee J R; Yasuda
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     Scherer S W; Saheki T
    Department of Biochemistry, Faculty of Medicine, Kagoshima University,
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     Japan.. dodoko12@med2.kufm.kagoshima-u.ac.jp
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     Highly conserved charge-pair networks in the mitochondrial carrier family.
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     Nelson D R; Felix C M; Swanson J M
ΑU
     Department of Biochemistry, The University of Tennessee, Memphis, TN
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     38163, USA.
     R01-HL54248-02 (NHLBI)
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     Sun J; Rhodes J C; Askew D S
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     Department of Pathology & Laboratory Medicine, University of Cincinnati,
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     OH 45267-0529, USA.
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      Liu Q; Dunlap J C
      Department of Biochemistry, Dartmouth Medical School, Hanover, New
 CS
      Hampshire 03755, USA.
      GM-34985 (NIGMS)
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      MH-01186 (NIMH)
      MH-44651 (NIMH)
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EM 199702

ED Entered STN: 19970219

Last Updated on STN: 19970219 Entered Medline: 19970206

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DUPLICATE 1 ANSWER 1 OF 11 MEDLINE L3 Uncoupling proteins (UCPs) are members of the mitochondrial transporter AB family that dissipate the proton gradient as heat more than via ATP synthesis. In the present study, nucleotide and amino acid sequences of UCPs 1, 2 and 3 of a dog were determined, and their mRNA expression in various peripheral tissues was examined. The sequences were highly (76-97%) homologous to those of other species. Although lower homologies (60-74%) were found when compared among the three canine UCPs, their deduced amino acid sequences had some common domains, such as three mitochondrial carrier protein motifs, six transmembrane alpha-helix domains, and putative purine nucleotide binding domains. By Northern blot analyses, UCP1 mRNA was not detected in any tissues examined. UCP2 mRNA was expressed in most tissues, particularly abundantly in adipose tissue, spleen and lung. Two sizes of UCP3 mRNA were found exclusively in heart and skeletal muscle. These results suggest that canine UCPs have uncoupling activity, and are involved in the regulation of metabolic heat production and/or energy expenditure, as do those of other species.

L3 ANSWER 2 OF 11 MEDLINE

DUPLICATE 2

The mitochondrial aspartate/glutamate carrier catalyzes an important step AB in both the urea cycle and the aspartate/malate NADH shuttle. Citrin and aralar1 are homologous proteins belonging to the mitochondrial carrier family with EF-hand Ca(2+)-binding motifs in their N-terminal domains. Both proteins and their C-terminal domains were overexpressed in Escherichia coli, reconstituted into liposomes and shown to catalyze the electrogenic exchange of aspartate for glutamate and a H(+). Overexpression of the carriers in transfected human cells increased the activity of the malate/aspartate NADH shuttle. These results demonstrate that citrin and aralarl are isoforms of the hitherto unidentified aspartate/glutamate carrier and explain why mutations in citrin cause type II citrullinemia in humans. The activity of citrin and aralar1 as aspartate/glutamate exchangers was stimulated by Ca(2+) on the external side of the inner mitochondrial membrane, where the Ca(2+)-binding domains of these proteins are localized. These results show that the aspartate/glutamate carrier is regulated by Ca(2+) through a mechanism independent of Ca(2+) entry into mitochondria, and suggest a novel mechanism of Ca(2+) regulation of the aspartate/malate shuttle.

L3 ANSWER 3 OF 11 MEDLINE

DUPLICATE 3

We cloned a novel mouse gene that encodes a protein with homology to the mitochondria solute carrier proteins (Mscp). The major full-length Mscp transcript contains 4112 bp of cDNA and a deduced protein of 338 amino acids. The Mscp protein shares 50%, 40%, and 39% sequence identity with the C. elegans hypothetical protein T26089 and the yeast mitochondria carrier proteins MRS3 and MRS4, respectively. It also showed homology with the uncoupling proteins (UCP1, UCP2, and UCP3; 22%, 24%, and 29% identity, respectively). The protein has six transmembrane domains and three mitochondria energy-transfer protein signature motifs, which are conserved among all the members of mitochondria carrier protein family. Northern analysis indicated that the Mscp gene is highly expressed in the spleen. Using cDNA microarray and Northern analysis, we have shown a significant decrease of the splenic Mscp mRNA levels around 4-5 weeks of age in several mouse strains including C57BL/6J, nonobese

OD-congenic mice. These result uggest that diabetic (NOD), and severa the Mscp gene is decreased during splenic lymphocyte maturation in these mice.

ANSWER 4 OF 11 HCAPLUS COPYRIGHT 2002 ACS L3

A cDNA clone (DNA-80562-1663) has been identified, that encodes a novel AB protein having homol. to known human uncoupling proteins. The protein of invention was designated as "uncoupling protein 5" or "UCP5". A signal peptide, a tyrosine kinase phosphorylation site, N-myristoylation sites, three mitochondrial carrier protein motifs were identified in UCP5. Three human isoforms of UCP5 (UCP5L, UCP5S, and UCP5SI) were identified; as well as two mouse isoforms (UCP5L and UCP5S). The human UCP5 gene has been mapped to chromosome X (q23-q25). Also provided herein are vectors and host cells comprising those nucleic acid sequences, chimeric polypeptide mols. comprising the polypeptides of the present invention fused to heterologous polypeptide sequences, antibodies which bind to the polypeptides of the present invention, and methods for producing the polypeptides of the present invention.

ANSWER 5 OF 11 HCAPLUS COPYRIGHT 2002 ACS L3

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Uncoupling proteins (UCP) found in the inner mitochondrial membrane of mammals dissipate the proton electrochem. gradient across the inner membrane to produce heat rather than synthesize ATP. Using PCR-based methods, we isolated two novel cDNA clones, WhUCP1a and WhUCP1b, that encode the mitochondrial uncoupling protein of wheat (Triticum aestivum L.). The cDNA clones each contain one ORF which can code for a protein of 286 amino acids with a predicted mol. mass of about 30.5 kDa, although three amino acid substitutions are found between them. The deduced amino acid sequences each possess three typical mitochondrial carrier signature domains and six membrane-spanning domains which are highly conserved in the mitochondrial transporter family. Southern anal. suggested that the WhUCP1 gene may be present in as many as three copies in the wheat genome, and also that WhUCP proteins may be encoded by a small multigene family. Northern anal. revealed that the steady-state level of the WhUCP1 mRNA is quite low. Quant. RT-PCR clearly showed that expression of the WhUCP1 gene in wheat seedlings is insensitive to low temp. Our data suggest that WhUCP1 might have functions other than low temp. -induced thermogenesis, although WhUCP1 possesses all the typical features reported for known

DUPLICATE 4 MEDLINE ANSWER 6 OF 11 L3

The Aspergillus nidulans acuH gene, required for growth on acetate and long-chain fatty acids, was cloned by complementation of the acuH13 mutation. Northern blotting analysis showed that transcription of the acuH gene occurs in acetate-grown mycelium and at higher levels in oleate-grown mycelium, but not during growth on glucose minimal medium. The acuH gene encodes a protein of 326 amino acids that belongs to the mitochondrial carrier family. The ACUH protein contains three related segments of approximately 100 amino acids in length, each segment comprising two hydrophobic domains that are probably folded into two transmembrane alpha-helices linked by an extensive polar region. Sequence comparisons suggest that the acuH gene of A. nidulans encodes the homologue of the carnitine/acylcarnitine carrier of rat and man. The uncharacterised proteins YOR100C of Saccharomyces cerevisiae, COLT of Drosophila melanogaster, and DIF-1 of Caenorhabditis elegans also seem to be homologues of ACUH. In addition to the motifs present in all members of the mitochondrial carrier family, we propose the highly conserved motif R(A,S)(V,F)PANAA(T,C)F within the sixth hydrophobic domain of these proteins as the characteristic feature of the carnitine carrier subfamily. The proposed function of the ACUH protein is the transport of acetylcarnitine molecules from the cytosol to the mitochondrial matrix, a process required during growth on acetate or on long-chain fatty acids.

DUPLICATE 5 ANSWER 7 OF 11 MEDLINE The human uncoupling protein 3 gene generates two mRNA transcripts, uncoupling protein 3L and uncoupling protein 3S, which are predicted to encode long and short forms of the uncoupling protein 3 protein, respectively. While uncoupling protein 3L is similar in length to the

hs, uncoupling protein 3S lack. The last 37 other known uncoupling pro C-terminal residues. A splice site mutation in the human uncoupling protein 3 gene, resulting in the exclusive expression of uncoupling protein 3S, and a number of point mutations in the uncoupling protein 3 gene have been described. This study compares the biochemical activity of uncoupling protein 3S as well as three mutants of the uncoupling protein 3 gene (V9M, V102I, R282C) with that of uncoupling protein 3L utilizing a yeast expression system. All proteins were expressed at similar levels and had qualitatively similar effects on parameters related to the uncoupling function. Both uncoupling protein 3S and uncoupling protein 3L decreased the yeast growth rate by 35 and 52%, increased the whole yeast basal 02 consumption by 26 and 48%, respectively, and decreased the mitochondrial membrane potential as measured in whole yeast by uptake of the fluorescent potential-sensitive dye 3'3-dihexyloxacarbocyanine iodide. In isolated mitochondria, uncoupling protein 3S and uncoupling protein 3L caused a similar (33 and 35%, respectively) increase in state 4 respiration, which was relatively small compared to uncoupling protein 1 (102% increase). A truncated version of uncoupling protein 3S, lacking the last three C-terminal residues, Tyr, Lys and Gly, that are part of a carrier motif that is highly conserved among all mitochondrial carriers, had a greatly reduced uncoupling activity. The two naturally occurring uncoupling protein 3 mutants, V9M and V102I, were similar to uncoupling protein 3L with respect to effects on the yeast growth and whole yeast 02 consumption. The R282C mutant had a reduced effect compared to uncoupling protein 3L. In summary, uncoupling protein 3S and the three mutants of uncoupling protein 3 appear to be functional proteins with biochemical activities similar to uncoupling protein 3L, although uncoupling protein 3S and the R282C mutant have a modestly reduced function.

DUPLICATE 6 MEDLINE ANSWER 8 OF 11 L3Citrullinaemia (CTLN) is an autosomal recessive disease caused by AB deficiency of argininosuccinate synthetase (ASS). Adult-onset type II citrullinaemia (CTLN2) is characterized by a liver-specific ASS deficiency with no abnormalities in hepatic ASS mRNA or the gene ASS (refs 1-17). CTLN2 patients (1/100,000 in Japan) suffer from a disturbance of consciousness and coma, and most die with cerebral edema within a few years of onset. CTLN2 differs from classical citrullinaemia (CTLN1, OMIM 215700) in that CTLN1 is neonatal or infantile in onset, with ASS enzyme defects (in all tissues) arising due to mutations in ASS on chromosome 9q34 (refs 18-21). We collected 118 CTLN2 families, and localized the CTLN2 locus to chromosome 7q21.3 by homozygosity mapping analysis of individuals from 18 consanguineous unions. Using positional cloning we identified a novel gene, SLC25A13, and found five different DNA sequence alterations that account for mutations in all consanguineous patients examined. SLC25A13 encodes a 3.4-kb transcript expressed most abundantly in liver. The protein encoded by SLC25A13, named citrin, is bipartite in structure, containing a mitochondrial carrier motif and four EF-hand domains, suggesting it is a calcium-dependent mitochondrial solute transporter with a role in urea cycle function.

DUPLICATE 7 ANSWER 9 OF 11 MEDLINE L3Selection for regain-of-function mutations in the yeast ADP/ATP carrier AB AAC2 has revealed an unexpected series of charge-pairs. Four of the six amino acids involved are found in the mitochondrial energy transfer motifs used to define this family of proteins. As such, the results found with the ADP/ATP carrier may apply to the family as a whole. Mitochondrial carriers are built from three homologous domains, each with the conserved motif PX(D,E)XX(K,R). Neutralization of the conserved positive charges at K48, R152 or R252 in these motifs results in respiration defective yeast. Neutralization of the negative charges at D149 and D249 also make respiration defective yeast, though E45G or E45Q mutants are able to grow on glycerol. Regain of function occurs when a complementary charge is lost from another site in the molecule. This phenomenon has been observed independently eight times and thus is strong evidence for charge-pairs existing between the affected residues. Five different charge-pairs have been detected in the yeast AAC2 by this method and three more can be predicted based on homology between

the domains. The highly concreted charge-pairs occurring with or between the three mitochondrial energy transfer signatures seem to be a critical feature of mitochondrial carrier structure, independent of the substrates transported. Conformational switching between alternative charge-pairs may constitute part of the basis for transport.

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DUPLICATE 8 MEDLINE L3 ANSWER 10 OF 11 Mitochondrial carrier proteins comprise a superfamily of evolutionarily AB conserved proteins that regulate the specific transport of essential metabolites across the mitochondrial membranes. In this report we describe the cloning and sequencing of a gene from Aspergillus nidulans, amc-1, that encodes the first reported example of a mitochondrial carrier protein in Aspergillus species. The amc-1 gene is located on chromosome 7, and is transcribed as a 1.6 kb unspliced polyadenylated RNA. The predicted translation product of the amc-1. cDNA displays three tandemly repeated domains which possess protein signature motifs that are characteristic of mitochondrial carrier proteins that localize to the inner mitochondrial membrane. amc-1 shares the greatest similarity with a Neurospora mitochondrial carrier protein that is implicated in basic amino acid transport, suggesting that the amc-1 protein may provide a related function.

DUPLICATE 9 MEDLINE L3ANSWER 11 OF 11 Mutations in arg-13 result in slow growth in minimal medium and can AB suppress mutations in carbamyl phosphate synthase-aspartate carbamyl transferase within the pyrimidine pathway; the exact biochemical function of the gene product is unknown. To understand the role of arg-13 in arginine metabolism, cosmids rescuing growth in arg-13 mutants were cloned and mapped to the position of arg-13 on LG IR. Northern analysis showed the arg-13 message to contain approximately 2100 nt, although a 1.4-kb genomic fragment truncated at the 5' and 3' ends of the gene encodes a shortened transcript that can rescue arg-13 function. Expression of mRNA arising from the mutant arg-13 gene is induced by arginine starvation, although wild type (arg-13+) is not derepressed in minimal medium. The sequence of the arg-13 gene shows ARG-13 to be a member of the mitochondrial carrier superfamily with three repeats of a approximately 100-amino acid domain, six putative membrane spanning regions, and three copies of the mitochondrial carrier consensus pattern. This information plus available and new nutritional data are consistent with the hypothesis that arg-13 encodes a mitochondrial basic amino acid carrier whose existence was predicted based upon previous physiological, nutritional and biochemical data.

L6 ANSWER 1 OF 67 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.

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AU Klingenberg M.

CS M. Klingenberg, Institute of Physical Biochemistry, University of Munich, Schillerstrasse 44, D-80336 Munich, Germany. klingenberg@pbm.med.uni-muenchen.de

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